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NUTRITION REVIEWS

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NUTRITION REVIEWS

VOL. 18 JANUARY 1960 No. 1

THE FOOD AND DRUG ADMINISTRATION FACES NEW RESPONSIBILITIES

Nearly 100 years ago a young Oxford mathematics instructor named Charles Lutwidge Dodgson published a children's classic under the pseudonym Lewis Carroll. Its publication resulted from a fantastic tale he told one afternoon to entertain Alice Liddell, one of the young daughters of the dean of the college (*Alice's Adventures in Wonderland* (*1865*)). In this fantasy Alice experienced many exciting adventures. But if a mid-nineteenth century Alice were to visit a modern American supermarket she would be just as amazed at the wonderful things she would see as she was in her trip down that imaginary rabbit hole in 1865. For she would indeed be in a wonderland of strange, beautiful, delicious, and attractively packaged foods from all parts of the world. She would find fresh, canned, frozen, dried, bottled, and plastic-wrapped foods unheard of in her day. She would also see pre-mixed, precooked, and ready-baked delicacies almost beyond belief.

But the chemical industry and modern food technology have made the job of the food and nutrition chemist also a strange wonderland—a wonderland of flavors, colors, emulsifiers, pesticide residues, conditioning agents, enzymes, gas propellants, antioxidants, vitamins, amino acids, and preservatives. Faced with the ever changing composition of American foods, a chemist in the laboratories of the Food and Drug Administration can truly say, as the Queen said to Lewis Carroll's Alice: "Now here, you see, it takes all the running you can do to stay in the same place" (*Through the Looking-Glass* (*1870*)).

Research in Nutrition and Foods. Research has not been one of the major activities of the Food and Drug Administration. This is not through lack of interest, but because the heavy demands of routine analyses for enforcement purposes have required most of the limited laboratory space, personnel time, and money. Nevertheless, the various divisions have made significant contributions to the literature of foods and nutrition. With only limited funds available for research, it would be expected that the type undertaken would be largely in analytical methodology, and such has been the case. The Division of Food has been especially active in developing the collaborative studies of the Association of Official Agricultural Chemists. As a matter of fact, the Journal of the A.O.A.C. is an unofficial organ of the Food and Drug Administration.

The late Elmer M. Nelson, who at the time of his unexpected death in late 1958 was Director of the Division of Nutrition, was one of a few outstanding leaders in the scientific work of the FDA and its antecedent organizations. He was recognized for his statesmanship in national and international matters dealing with vitamin assay methods and control. It is most fitting, therefore, that it would be his laboratory which developed a chemical method for vitamin D assay to replace the tedious and inexact rat bio-assay method (J. B. Wilkie, S. W. Jones, and O. L. Kline, *J. Am. Pharm. Assn.* **47,** *385* (*1958*)).

Although work still remains to be done to gain official approval for this method by the U.S.P. and the A.O.A.C., it should ultimately result in great saving of time in the vitamin D_2 analysis of pharmaceuticals and possibly also of foods. Unfortunately it cannot yet be used for the assay of vitamin D_3, so the chick must still be used for this assay.

It can be said truly that the passing of Elmer M. Nelson marks the end of an era, an era which identified vitamins with rats.

Special Dietary Foods. Under the "403

(j)" section of the Federal Food, Drug, and Cosmetic Act, the Food and Drug Administration has authority to promulgate regulations for interstate commerce in foods which are purported to have special dietary uses. This much discussed part of the law reads:

"SEC. 403. A food shall be deemed to be misbranded—

(j) If it purports to be or is represented for special dietary uses, unless its label bears such information concerning its vitamin, mineral, and other dietary properties as the Secretary [of Health, Education, and Welfare] determines to be, and by regulations prescribes as, necessary in order fully to inform purchasers as to its value for such uses."

Consonant with this authority, regulations regarding the labeling of vitamin content and mineral content of foods have been established.

Also under this authority the Administration has established values for the "minimum daily requirements" of man for various of the vitamins. The fact that these are *minimum* requirements and are therefore lower than the Recommended Dietary Allowances of the Food and Nutrition Board of the NAS-NRC (*Publication 589, National Academy of Sciences-National Research Council (1958)*) has caused no small amount of confusion, even among experts.

One of the most controversial regulations promulgated under the 403 (j) section of the Act prescribes the required label declaration for artificially sweetened foods. Such labels must contain the words: "Contains ——— . . . , a non-nutritive, artificial sweetener which should be used only by persons who must restrict their intake of ordinary sweets." The wording of this declaration was written when saccharin-sweetened foods were designed primarily for diabetics. Since then the significance and extent of obesity as a serious American health hazard has caused widespread manufacture, sale, and use of artificially sweetened foods for weight reduction or control. Such use of purported "low-calorie" foods has greatly exceeded their use by diabetics, and since weight reduction is often attempted by the do-it-yourself technique, the wording ". . . who *must* restrict their intake of ordinary sweets" becomes rather unsuitable. Suggestions have been made to modify somewhat this exact wording.

Enforcement of the Food, Drug, and Cosmetic Laws. Although most food, drug, and cosmetic manufacturers and producers do their best to supply the American people with safe, pure, wholesome materials at a fair price, there are occasional producers and dealers who are either ignorant, careless, stupid, or downright dishonest. Through long years of patient education and publicity, the Food and Drug Administration has elicited the cooperation of agriculture and industry so that legal actions against violation of the laws and regulations are only rarely necessary. Where misrepresentation, misbranding, short weight, contaminated or decomposed food, or dangerous drugs are found, and where voluntary correction cannot be obtained, the Department does not hesitate to use every legal means at its disposal.

The routine work of the Food and Drug Inspector is usually quite prosaic and unexciting. He inspects production areas, whether farm or factory; examines the premises for poor sanitary practices and sources of rodent or insect contamination; inspects raw materials and production methods; collects samples of foods, drugs, and cosmetics and their ingredients for chemical and microbiological examination; compares the label claims of the packaged materials with the ingredients found in the factory or warehouse; and, where indicated, suggests corrective action to alleviate poor production or manufacturing practices. The samples he collects are sent either to a district laboratory or to the headquarters laboratories in Washington for examination and analysis.

The laboratories of the Administration examine the collected specimens for decomposition, insect and rodent filth; analyze the foods for pesticide residues, vitamins, minerals, and other incidental or intentional additives; and compare the analytical data with the label claims and with the regulatory requirements. Thousands of such samples are analyzed each year on both domestic and imported foods, drugs, and cosmetics and the raw commodities going into their manufacture. The fact that very few of these sample examinations lead to legal action attests not only to the honesty and integrity of the great majority of the producers, but also to the effectiveness of the scientific and enforcement activities of the Food and Drug Administration.

Occasionally outright dishonesty or racketeering is uncovered. One recent case has all the exciting characteristics of a pulp magazine novel. It has been known for a number of years that incubator-reject rotten eggs were finding their way into foods, but evidence which could lead to a conviction was difficult to obtain. Anyone can tell a rotten egg from a fresh egg by taste and smell, but an odor cannot be given a quantitative value and is not readily captured to be presented later to a judge or jury. Also, it is possible to deodorize rotten eggs or to disguise their odor by mixing them with fresh eggs in fluid egg containers. Painstaking chemical research charted the changes which occur when eggs decompose and those data were found reliable in giving objective evidence of decomposition. In large hatcheries it is common practice to "candle" the incubating eggs at the twentieth day, and those which are not viable are removed and discarded. There is little legal outlet for such rejects, so some unscrupulous people have at times collected, reconditioned, and sold them to food manufacturers and bakers as edible broken, fluid eggs.

In a recent case, evidence was obtained that rotten eggs from certain hatcheries were being diverted to illegal food use. By the device of an invisible ultraviolet-fluorescent dye, certain cases of the reject eggs were marked for later identification. When the eggs were picked up in the middle of the night by a racketeer's truck, the FDA inspectors with the assistance of local agents followed the truck at high speed through several states. The chase took several days and nights. Finally, the drivers, suspecting that they were being followed, abandoned the truck. But the inspectors continued their surveillance and finally caught the drivers, and, through them, the gangsters responsible. The chain of evidence brought a conviction and so, temporarily at least, this foul practice has been stopped.

The regulatory activities of the FDA include not only food, drug, and cosmetic inspection and control, but also the certification of certain food and drug materials, including certifiable coal-tar dyes, antibiotics, and insulin preparations.

Food Standards. Federal laws authorize the FDA to establish standards for foods, and such standards have been promulgated for a large number of foods. These specify the type and amount of required ingredients, list certain optional ingredients which may be used, and regulate the labeling permitted. For some types of foods, standards of fill of container have been issued to prevent the packer from using a deceptively large container or substituting water or sugar solution for the food product. Standards of quality have also been published for some fruits and other foods, so that substandard materials cannot be represented as that of first quality. Substandard food can be sold with appropriate labeling but the alert purchaser will know what he is getting.

Most of the standards are referred to as Definition and Standard of Identity. Adherence to these standards assures the consumer that he will receive a nutritious, safe product, informatively labeled. Unfortunately, the standards are worded in a

legal-scientific jargon which is difficult for the average person to understand, and information about these standards has not been made readily available to the average consumer. Much of the value of the food standards is thus lost because of public ignorance of their significance.

Foods which are not standardized are required to be labeled with a list of ingredients, identified by common or usual names, and, although the percentage composition of the product is not required, it is mandatory that the ingredients be listed on the label in descending order of importance, percentage-wise. This aspect of labeling requirement is probably not as widely known as would be desirable for consumer protection and information.

Nutrition Education. One continuous responsibility of the FDA is the campaign against food faddism and nutritional quackery. The fraudulent purveyor of aloe leaves, papaya, seaweed, dried alfalfa, pega palo, or royal jelly—who through ignorance or cupidity makes fantastic claims for his product—can often be dealt with through legal means. The American Medical Association and the Better Business Bureaus are staunch allies of the Government in these cases. But the education of the general public in sound nutrition practice is a matter which demands the full cooperation of every agency of education and government. Members of the staff of this agency, from the Commissioner himself down to the field office inspector, are constantly teaching the principles of good nutrition, both in public addresses and private conversation.

The dramatic successes of nutrition science in eradicating certain formerly widespread deficiency diseases in America have convinced a large segment of the population that *any* human ailment can be cured if one could only find the right vitamin, mineral, protein, amino acid, or combination of them. This is of course nonsense, but the impression prevails. It is therefore not surprising that many unsophisticated people fall prey to the self-styled nutrition expert who is willing to claim anything for his product, whether it be a vitamin pill or dried alfalfa.

The Safety of Food Additives. The term "food additive" may include flavors, pesticide residues, artificially induced radioactivity, antioxidants, emulsifying agents, packaging materials, enzymes, conditioners, microbial inhibitors, preservatives, and many other substances. The amendment to the food and drug laws passed by Congress in 1958—commonly referred to as the Food Additive Amendment—was designed to, and does, place upon the *manufacturer* the legal responsibility for proving the safety of a food additive. Under the previous law, an additive could be banned as harmful only if the Food and Drug Administration obtained proof of harmfulness. With hundreds of chemicals and mixtures of unknown composition being added to foods, it was patently impossible for the FDA scientists to screen more than a few of these additives for safety, with the niggardly funds made available by Congress for this purpose.

This shift in the responsibility for proof of safety of a food additive from the Government to the manufacturer would seem, superficially, to relieve the Food and Drug Administration scientists of a major load of work. In actual fact, however, it adds a stupendous burden because it makes it all the more essential that the FDA have a highly competent corps of specialists in biochemistry, biophysics, physiology, pharmacology, toxicology, and pathology. The toxicological examination of a substance is not a straightforward procedure; there are no simple guidelines—every potentially toxic material is a new problem. And evaluating the data submitted by a manufacturer in support of the safety of an additive is not a matter which can be turned over to a clerk or a computer.

Only experts in the various physical, chemical, biological and medical sciences

have such competence. To have attained the judgment required, these scientists must themselves have been engaged in fundamental research in their respective disciplines, and to maintain this competence they must have the time, the resources, and the freedom to be continuously engaged in basic research. Unfortunately, Congress has not yet s the necessity of making funds available is essential aspect of the enforcement of the Food Additive Amendment.

The Federal Government spends less than seven cents per person each year for protection of foods, drugs, and cosmetics against adulteration, misbranding, filth and decomposition; protection against dangerous drugs and cosmetics and the hazards of pesticide residues and radioactivity; and all the other enforcement and research activities of the Food and Drug Administration. This amounts to less than the cost of one candy bar per person per year. Expressed in another way, Congress appropriates to the Food and Drug Administration each year less than one-sixth of the cost of one military jet bomber.

It is my earnest hope that the Food and Drug Administration will immediately enter into a new, aggressive phase of development in which the essential basic research needed in all aspects of food and nutrition will become a major enterprise. This will require a fundamental change in viewpoint within the organization, and the active consent of Congress as expressed by adequate appropriations.

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VITAMIN A TOLERANCE TEST AND DISORDERS OF FAT METABOLISM

Vitamin A tolerance tests were applied to 54 anginal patients. High values for vitamin A paralleled fats but not cholesterol. Coronary atherosclerosis occurred in several types of lipid disorders.

Since coronary atherosclerosis accounts for so many deaths in our society, it is fitting that investigators are seeking avidly for a means of detecting some metabolic defect which, in susceptible persons, may provide means for prophylactic management. Some measure of success has been achieved in the statistical relationships between coronary disease and increased blood levels of cholesterol and beta-lipoprotein. Further information has come from the use of test meals to measure absorption and utilization of lipids. Total lipids, chylomicrons, unesterified fatty acids, glycerol, triolein and radioactive fats have been studied in this manner, but none is a perfect test.

Vitamin A was employed by J. L. Beaumont and J. Lenègre (*Am. Heart J.* **58,** *163 (1959)*) to study the tolerance of 54 anginal patients to fat. The authors reported that vitamin A esters behave as a tracer of neutral fat during the first stages of its metabolism.

A neutral vitamin A in oil was given to the subjects as follows: 100,000 units at 9:00 a.m. on the first and second days, none on the third day, and 500,000 units on the fourth. Breakfast was fed immediately after the test dose. Blood was collected on the first and fourth days before the test dose and at three, six, nine, 12 and 24 hours thereafter. The normal ranges for the vitamin A tolerance test were established in a group of 19 patients with cardiac disease but without atherosclerosis.

The 54 patients were divided into the six following groups: seven with primary

hypercholesterolemia, eight with essential hyperlipemia, two with diabetes mellitus and hyperlipemia, one with myxedema, 24 with atypical and usually moderate hyperlipemia, and 12 with normal fasting blood lipids.

The vitamin A tolerance test gave normal values for patients with essential hypercholesterolemia, and normal to elevated values for those with normal fasting blood lipids. However, the test gave abnormally high values for the patients with essential hyperlipemia, and variable results for those subjects with atypical hyperlipemia.

The authors interpreted these data as indicating a group of different metabolic disorders which have in common a disturbance of lipid metabolism and coronary artery disease. The vitamin A tolerance test seemed to be a means of identifying those subjects who had a disturbance of the preliminary stages of lipid metabolism (absorption, transportation and storage). There was a notable lack of correlation with the serum cholesterol.

The vitamin A tolerance test would appear to be a useful device for further investigation of lipid metabolism, especially in groups of patients who have alterations in rates of absorption, transportation and storage of neutral fats.

THE NORRBOTTEN STUDY

The relationship between breast and bottle feeding on growth, ossification, antibody responses and serum electrolytes has been studied in a group of 402 infants in northern Sweden.

There are many serologic and biochemical differences between human milk and cow's milk. The way in which these differences influence the rapid growth of small infants has become of increasing interest in the last few years, although formerly the differences in chemical composition were thought to have little effect. Therefore, in order to reassess the clinical, chemical and immunologic differences between these two types of milk, a large field study was organized in Norrbotten County in the north of Sweden by O. Mellander, B. Vahlquist, T. Mellbin and collaborators (*Acta Paediat.* **48,** *Suppl. 116 (1959)*). Their specific aim was to evaluate the influence of these two types of feeding on infection and skeletal development.

The County of Norrbotten lies at the Arctic Circle and the subjects studied were from a county district and a mining town. There were no measurable differences between the two groups and the results were combined. All the children in the study, as well as their mothers during pregnancy, were under strict health supervision. Between June 1, 1953, and December 31, 1954, there were 660 births in the two areas studied. Four-hundred and two of these infants completed the total program and are the basis of this report.

The general health and dietary habits of the population were satisfactory and no quantitative or qualitative evidence of malnutrition was known to occur. The diets of all the expectant mothers were fortified with animal protein, iron and vitamins and an attempt was made to provide optimum conditions for the development of the fetus. The infants were seen by the public health nurse every two to four weeks during most of the study and had medical examinations every six to twelve weeks. Blood samples for serological and biochemical analysis were obtained at birth, at seven and a half months of age, and again at 30 months of age. The immunization program consisted of tetanus, diphtheria and pertussis at three, four and a half, and six months of age and influenza vaccine at seven months. Skeletal x-ray examinations were made at seven and a half and 30

months. In addition a dental examination was carried out at 30 months of age.

The incidence of full breast feeding for these groups fell from 80 per cent at birth to 32 per cent at six months of age. Supplemental feeding or wholly artificial feeding consisted of a mixture of half cow's milk and half water with 5 per cent sugar and 1 per cent wheat flour. This mixture gave 550 calories per liter and contained 1.7 per cent protein, 1.5 per cent fat, 8 per cent carbohydrate and 0.4 per cent ash. The intake was limited to 600 to 700 g. at one month, increasing to 800 to 1000 g. at three months. Crushed rusks were added at four months, combined with fresh cow's milk for one group and a commercial dried milk preparation for another. From the age of one week the diet was supplemented daily with 2500 units of vitamin A and 1000 units of vitamin D, and this dose was doubled at three months at which time 25 mg. of ascorbic acid were added. Strained baby foods were introduced at four months and the mothers were given a free hand in feeding after the seven and a half-month examination.

The infants were divided into four groups according to the duration of breast feeding. Group one was at breast for less than two weeks, group two from one to two and a half months, group three from three to six months, and group four for six and a half months or longer. Special attention was given to group one with little or no breast feeding and group four which had been exclusively breast fed for over six months. Systematic analysis of the family size, age of mothers, economic status, general health, nutrient intake during pregnancy, birth weight, season of birth and sex of the four groups failed to reveal any statistically significant differences.

Clinical examination, however, revealed a number of differences between groups one and four. The weight gain at seven and a half months was greater for the artificially fed infants than for the breast fed infants, the gain on the cow's milk formula being 5.52 kg. while on the breast milk it was 4.90 kg. This difference was statistically significant at a probability of .001 or less and at 30 months was somewhat smaller ($P = .05$ to .01). There was also a small but significant difference in height between groups one and four at seven and a half months, the bottle-fed infants being taller, but this disparity disappeared at 30 months of age. In the bottle-fed female infants at seven and a half months there were more ossification centers than in their breast-fed counterparts, but, again, this disparity disappeared at 30 months. The only significant differences for hemoglobin and sedimentation rates were at three months of age when there was a slightly lower hemoglobin and slightly higher sedimentation rate in the bottle-fed infants.

Clinical observation and serologic studies revealed no differences in the incidence of fever or the common communicable diseases. However, the detailed clinical records kept on the incidence of rhinitis, cough, otitis media, upper respiratory infection with fever, and acute diarrhea showed that these five infections were more frequent in the country district. There were a few significant differences between feeding groups, the incidence of these infections being slightly higher for the bottle-fed infants. However, the probability of statistical significance never exceeded the 5 per cent level. When the infants of group one were compared with those for group four for all the infections and for all age groups of the first year, those weaned from the breast during the first two weeks of life had significantly more infections than those of the exclusively breast-fed group with a probability of .01 or less. The authors carefully point out that these data apply only to the incidence of acute infections and that they were unable to determine either the severity or the duration of these infections.

The immunologic studies consisted of antistreptolysin and antistaphylolysin titers measured at seven and a half and 30 months

of age, but no significant differences between feeding groups were found.

The response to immunization was studied by measuring antibody response to diphtheria, pertussis and influenza B antigens. Again no correlation with feeding groups was found.

At seven and a half and 30 months of age biochemical assessment was made of total serum proteins, electrophoretic pattern, calcium, phosphorus, alkaline phosphatase, and sodium and potassium in serum. The gamma globulin levels in groups one, two and three were significantly higher than in group four, and it should be noted that the higher gamma globulin levels were in those groups having a slightly higher incidence of infection but which had received cow's milk, whereas group four had received much less cow's milk during the first six months of life. There were no other differences of importance in the electrophoretic pattern.

The serum calcium concentrations in the exclusively breast-fed group four were significantly higher than in group one, indicating that the serum calcium does not have a direct relationship with the calcium intake. On the other hand, those receiving cow's milk (groups one, two and three) showed significantly higher levels of serum phosphorus than did group four, correlating well with the intake. An alternative explanation would be that these differences reflected a constant calcium-phosphorus product, since the alkaline phosphatase values were significantly higher in the infants receiving cow's milk. The similarity to the biochemical changes in rickets was only superficial since the serum phosphorus levels were also raised and x-ray examination of the bones revealed no sign of rickets in any child. No significant differences between the feeding groups were found respecting any of the other components studied.

Dental examination consisted of noting the time of eruption of the deciduous teeth and the incidence of caries at 30 months. The type of feeding had no influence on the time of eruption of the deciduous teeth, and there were no clear-cut differences in the overall incidence of caries among the feeding groups.

Summary:

In general the differences that were found seem clear-cut and to be solely due to the differences in feeding between the various groups, but, as might be expected, most of the differences were statistically significant only when comparisons of the extreme groups were made. Moreover, some of the differences, such as those attributed to growth and ossification centers, existed only for a limited period during early infancy. However, the data concerning the incidence of acute infections of the upper respiratory passages and the gastrointestinal tract are particularly pertinent since they tend to confirm previous observations made under poor conditions of hygiene. Small changes in the gamma globulin concentration were found, but differences in response to immunizations with the usual types of vaccines did not appear to be influenced by nutritional factors. The differences in serum calcium, phosphorus and alkaline phosphatase were noted only at seven and a half months, disappearing at 30 months, and could probably be attributed to the higher phosphorus intake since there was no evidence of rickets in any of these infants who received large doses of vitamin D. It is pointed out that the differences between bottle-fed and breast-fed infants in this particular study have meaning only for the specific cow's milk formula that was used, and cannot be applied to other types of cow's milk feeding.

This well-designed and carefully executed study will become a current classic in studies on infant feeding, and sets a very high standard for the design and execution of further studies on other types of cow's milk formulas.

CORONARY HEART DISEASE AND DIETARY HABITS

Statistics of coronary artery disease for 20 countries have been re-evaluated and a distinct association with high intakes of saturated fats and animal proteins has been found.

The dietary habits of our ancestors as well as ourselves have been given strong emotional and social importance. Foods of animal origin have signified strength, health and vigor, as well as security and affluence. Yet in reading through a sequence of 29 articles in *Nutrition Reviews* on the subject of coronary heart disease in relation to dietary habits, one gains the impression that coronary artery disease and atherosclerosis are more common in men or animals fed large quantities of fats, particularly those of animal origin (*Nutrition Reviews* **6** *176, 293 (1948)*; **7,** *155 (1949)*; **8,** *41, 74, 176, 203, 304 (1950)*; **11,** *72, 191 (1953)*; **12,** *25, 270, 325 (1954)*; **13,** *20, 138 (1955)*; **14,** *3, 95, (1956)*; **15,** *17, 353 (1957)*; **16,** *1, 102, 129, 263 (1958)*; **17,** *55, 62, 102, 147, 159 (1959)*.

What one does not find, however, is a statement of the progressively longer survival or better state of health of those same people who may endanger their health thrice daily at the dinner table. Are we magnifying a scientific measurement out of its true proportion? Dr. George V. Mann stated in his address at the recent A.M.A. Convention, "The evidence will not justify very low fat diets, very low cholesterol diets, the addition of lipotropic agents, the use of supplements of vitamin B_6, or the addition to diets of the so-called essential fatty acids" (*Prog. 108th Annual Meeting of the A.M.A., p. 87, (1959)*).

N. Jolliffe and M. Archer (*J. Chron. Dis.* **9,** *636 (1959)*) have evaluated data based upon records of the World Health Organization and the Food and Agriculture Organization. These data were collected from 20 countries and furnished comparisons between death rates from coronary heart disease and dietary as well as environmental factors.

The authors stressed that one must consider several factors in interpreting such information. First to be taken into account are the relative efficiencies of the individual data-gathering systems. Also, the amount of food available is a residual figure and the final value is therefore subject to the variables of production, import, export, animal consumption, non-food use and waste. And finally, the dietary data are for food available at the retail level and, therefore, do not exactly represent consumption figures. There could well be greater emphasis upon one of the most important uncertainties in the kind of data gathered, namely, the effect on fat utilization of an excessive intake of calories from any sources beyond actual need.

Next they reviewed the interpretations of others. A. Keys in his report (*J. Mt. Sinai Hosp.* **20,** *118 (1953)*) relied upon data from six countries, and showed a relationship between the proportion of total fat calories available in the national diet and the death rates from degenerative heart disease for men in selected age groups. J. Yerushalmy and H. E. Hilleboe considered all the countries for which adequate data were available, but did not consider the quality of the fats eaten (*N.Y. J. Med.* **57,** *2343 (1957)*).

The National Dairy Council (*Dairy Council Digest* **28,** *1 (1956)*) stated that "no relationship is apparent between incidence of arteriosclerotic and coronary heart disease and any element of diet listed." (The Council listed total calories, total protein, animal protein, total fat, animal fat, vegetable fat, per cent calories total

fat, per cent calories animal fat and per cent calories vegetable fat.)

The point stressed by Jolliffe and Archer is that none of these investigators considered the quality of fat (*i.e.* saturated *vs.* polyunsaturated) in their statistical evaluation. They felt that the relationship between coronary heart disease and the intake of saturated fats as implied by B. Bronte-Stewart (*Lancet* **I,** *521 (1956); Brit. Med. Bull.* **14,** *243 (1958)*), L. W. Kinsell (*Fed. Proc.* **14,** *661 (1955)*) and H. M. Sinclair (*Lancet* **I,** *381 (1956)*) must pass a rough test by showing general agreement with such data. Accordingly, these authors employed the United Nations data in order to indicate additional lines of investigation and to test the hypothesis that "quality of fat" is a factor in the incidence of coronary artery disease.

The countries studied included those evaluated by Yerushalmy and Hilleboe. The authors posed the following problem: can death rates from "degenerative heart disease" (including arteriosclerotic heart disease, chronic endocarditis, and other degeneration of the heart) for men aged 55 to 59 years from 20 countries, be related statistically to dietary data or to other factors?

They also evaluated "combined categories" of heart disease (vascular lesions affecting the central nervous system, chronic rheumatic heart disease, arteriosclerotic and degenerative heart disease, other diseases of the heart, and hypertension with mention of heart disease), and "arteriosclerotic heart disease" alone. These categories were compared statistically with total death rates, average total available calories, total fat intake, saturated fat intake, unsaturated fat intake, animal protein intake, and with a social factor, *i.e.*, the number of telephones per 100 persons.

Categorically, the authors considered fats to be saturated (eggs, chicken, sheep, beef, pig, cow's milk, cocoa bean, coconut, lard, tallow); monounsaturated (olive oil); or polyunsaturated (peanut, linseed, pecan, walnut, safflower, corn, cottonseed, sunflower, sesame seed, barley, wheat, rapeseed, marine mammals and teleosts). They compared each category of cardiac disease with each environmental factor. Furthermore, they considered more than one factor at a time to determine co-variance.

The results showed that the death rate from degenerative heart disease was related statistically to per cent of saturated fat (0.8259), per cent of animal protein (0.8016), per cent of fat as total calories (0.7321) and number of telephones per 100 persons (0.6901). A negative correlation existed when per cent of calories as unsaturated fat was considered (−0.3218).

Multiple correlations made it evident that some of the factors depended upon each other, *e.g.*, total per cent of fats as calories and total calories supplied by unsaturated fats. Actually little more of the variance was accounted for by considering more than one factor at a time. By means of beta coefficients they were able to compare the relative effects of each variable on a regression line and to determine the relative contribution of each factor to the correlation coefficient.

Thus they ascertained that the intake of saturated fats was the most important factor and the next most important was intake of animal protein.

They postulated that as the economic wealth and caloric intake of a country increase, this increase consists mostly of fat, particularly saturated fats which accompany animal proteins. The negative correlation found for polyunsaturated fats confirms this theory for, as animal foods become abundant, vegetable foods may be used less.

A significant correlation was found between the number of telephones and degenerative cardiac disease. The number of telephones also correlated with the consumption of saturated fats.

While specific information about arteriosclerotic heart disease alone was available

for only 14 of the 20 countries, and hence the significance of the data weakened, yet the correlation with consumption of saturated fats was so high (0.9136) that one can scarcely discredit its validity. The correlation with animal protein was second (0.8333) and paralleled that for the mixed category.

The authors recognized that some people believe the increase in coronary artery disease is an artifact produced by better diagnostic and recording systems, older age of patients and greater public awareness. Nevertheless, they considered it likely that a tenfold increase in incidence from one country to another was significant. Furthermore, the present investigation intended to determine why the present rate is so high, and not why or whether it has been increasing. Finally, the authors pointed out that the correlations here demonstrated are in accord with the results of laboratory investigations and hence are credible.

Unfortunately there is no adequate appraisal of what may be a very important relationship, namely, an unfavorable effect from habitually consuming more calories than are needed by a large proportion of the population. There are indications that chronic consumption of calories above expenditure requirements may be an important factor in its effect upon cholesterol regulation and atherosclerosis. A trend in this direction might well be correlated with the same positive factors recorded by the authors, including a high intake of total fats, animal fats, animal proteins, saturated fats and such socio-economic factors as the number of telephones.

Nevertheless, this study is a significant contribution to a field of investigation fraught with opinion, confusion and doubt. The authors have demonstrated a distinct correlation between arteriosclerotic heart disease and the eating of saturated fats and animal protein. Yet they do not claim that this is the only factor, nor do they suggest sweeping revisions of our nutritional pattern. Cool deliberation and critical investigation will in time provide a basis for intelligent advice.

HUMAN LIVER GLYCOGEN LEVELS

The glycogen content of liver biopsies from 37 individuals was determined and no relationship was noted between the glycogen level and the nutritional status as judged by body weight.

The level of liver glycogen, the major carbohydrate store of the body, depends upon the immediate nutritional history of the individual, providing there is no abnormality in the structure of the glycogen or in glycogen production. In various mammals the amount of glycogen in the liver varies between 2 and 8 per cent when based upon wet weight of the organ. Liver glycogen is a very labile substance and in laboratory animals a fast of 24 hours or longer will almost completely deplete the liver of its glycogen. While numerous values are given in the literature for the glycogen content of the liver of most experimental animals, information on the glycogen content of the livers of normal humans is not nearly as extensive.

Recently, however, J. E. Sokal and K. E. Gerszi (*J. Lab. Clin. Med.* **53,** *876 (1959)*) have reported on the glycogen contents of the livers of 37 individuals. In all cases the biopsies of liver were obtained during the course of either a diagnostic laparotomy or a surgical procedure, with great care taken to obtain normal biopsies, particularly in those cases where there was malignancy present. It should be noted that, while the preoperative nutrition and anesthesia (cyclopropane often supplemented by the muscle relaxor succinylcholine) were similar, all the patients had been fasting 14 hours prior to surgery.

This would automatically reduce the glycogen content of the liver.

During the preoperative period, the patients were given oral diets offering between 1400 and 1900 calories per day with 125 to 200 g. of carbohydrates. However, they did not always eat all that was given them.

Of the 37 patients studied, 31 had no evidence of jaundice and seven were jaundiced. In the nonjaundiced patients, it was found that the glycogen content of the liver expressed as grams of glycogen per 100 g. of liver was 2.22 ± 0.27, while the glycogen content of the livers of the seven jaundiced patients had a similer mean value of 2.12 ± 0.46. Between particular patients, however, the variation in the glycogen content of the liver was quite large, ranging from 0.34 to 5.98 g. per cent, but this variation could not be correlated with the patients' weight.

These investigators compared their values to those obtained by D. S. MacIntyre, S. Pedersen and W. G. Maddock (*Surgery* **10,** *716 (1941)*), N. F. Young, J. D. Abels and F. Homburger (*J. Clin. Invest.* **27,** *760 (1948)*) and J. A. Hildes, S. Sherlock and V. Walshe (*Clin. Sci.* **7,** *287 (1949)*). Analysis of variance failed to show heterogeneity among the four groups. MacIntyre and associates and Young and co-workers obtained liver samples during biopsy while surgical procedures were being carried out on anesthetized patients, while Hildes and associates obtained the specimens by needle biopsy from unanesthetized individuals. It should be noted that all investigators subjected the patients to at least an overnight fast before obtaining the biopsies. From the combined series of data it appears that under these conditions of moderate fasting the upper limit of liver glycogen is about 6 per cent.

The fact that glycogen has a very rapid turnover rate (so that the concentration of liver glycogen would be primarily determined by the nutritional events of the 24 to 72 hours preceding the biopsy) may explain why no relationship was noted between liver glycogen and the apparent nutritional status of the patients.

Although it has been reported that levels of glycogen in individuals with glycogen storage disease can go as high as 12 to 16 per cent, most of the values are below such a level and there may be considerable overlapping between the glycogen levels in this disease and individuals with normal liver glycogen. Both MacIntyre and associates and Young and co-workers reported that patients who were given large glucose feedings before obtaining the liver biopsy had glycogen levels ranging from 3.2 to 9.7 per cent.

In summary, these investigators have added quantitative data as to the glycogen content of the liver of normal patients who had been fasted overnight. While the glycogen content of nonfasted individuals would undoubtedly be higher, at present such data are not available.

NUTRITIVE VALUE OF PLANTS AS AFFECTED BY GEOGRAPHIC LOCATION

The environment of a plant may influence its nutritional value. Turnip greens from Blairsville, Georgia, possess vitamin B_{12} activity, absent in the same crop in Experiment, Georgia.

Both the atmosphere (or top environment) and the soil (or root environment) can influence the composition of plants (*Nutrition Reviews* **9,** *189 (1951)*; **12,** *310, (1954)*; **13,** *337 (1955)*). The importance to animal nutrition of such environmental influences has not received much attention, with the exception of well-known mineral deficiencies (phosphorus, cobalt, copper) or toxicities (molybdenum, sele-

nium) which have been noted in certain soil types and in certain areas of the world.

The reports of differences in animal growth response to turnip greens grown at several locations is, therefore, of interest (L. F. Gray, M. Speirs, and G. Matrone, *J. Nutrition* **63,** *345 (1957)*).

Corn, cowpeas, and turnip greens (crops widely used in human diets in many areas of the South) were produced from a common seed stock at three locations: Blairsville and Experiment, Georgia, and Raleigh, North Carolina. The corn and cowpeas were harvested at maturity and the turnip greens were harvested when full-grown but without blooms. The corn and cowpeas were stored in the harvested dry form. The turnip greens were dried at 55° to 60° C in a force draft oven, and were then deribbed and stored in air-tight containers.

The experiments were designed to study the influence of location on the nutritive quality of each of the three crops. To evaluate nutritive quality, a basal diet was fed weanling rats consisting of 40 per cent ground corn, 30 per cent ground cowpeas, 10 per cent ground turnip greens, 5 per cent fat, 1 per cent salt, and 14 per cent sucrose. Viosterol was given to each rat weekly by dropper. Any diet supplements were added to the basal diet at the expense of an equal weight of sucrose.

The basal diet was nutritionally incomplete since it supported growth in weanling rats only to the extent of about 60 per cent of that obtained on a normal stock diet. There was, however, a highly significant effect of location on the nutritive value of the turnip green component. In two different crop years the turnip greens from Blairsville, Georgia, were responsible for an approximate 20 per cent improvement in body weight gain when compared with the similar crop grown at Raleigh, North Carolina, or at Experiment, Georgia.

Additional studies were conducted in which diet supplements were used to determine the factors responsible for growth stimulation in the Blairsville crop.

Egg albumin, and methionine or vitamin B_{12} improved the diets containing turnip greens from either Blairsville or Experiment. Furthermore, these supplements eliminated the previously observed differences between the greens grown at the two locations. On the other hand, a vitamin mixture containing all of the B vitamins, except vitamin B_{12}, produced only a slight growth stimulation in the diet containing Blairsville turnip greens, but produced a significant response when added to the diet with Experiment turnip greens.

The difference in nutritive value of the turnip greens grown at the two locations was not caused by minerals, since the ash of one, when added to the diet containing the other, did not alter the nutritive value.

The results suggested, rather, that the basal diet employed was deficient in methionine, and that the vitamin B_{12} response could be explained by its sparing action on the methionine requirement. Microbiological assay for methionine indicated that the turnip greens grown at Blairsville contained approximately 4.5 mg. per gram (air-dry basis) while those grown at Experiment contained 3.4 mg. per gram. Moreover, a similar difference in total crude protein was noted in the same crop at the two locations.

Assay for vitamin B_{12} activity, using *Lactobacillus leichmannii* 4797 and *Ochromonas malhamensis* 11532, indicated a striking difference in favor of the turnip greens grown at Blairsville. These averaged about 7 millimicrograms per gram in vitamin B_{12} activity, contrasted with an average activity of less than 1 millimicrogram per gram for the crop grown at Experiment.

These results are of considerable interest since there are only two naturally occurring substances known which possess vitamin B_{12} activity for the animal and for *Ochromonas*, namely cyanocobalamin and vitamin B_{12III} (M. E. Coates and J. E. Ford, *The*

Biochemistry of Vitamin B_{12}, p. 36. Biochem. Society Sym. 13, Cambridge University Press, 1955). They are of particular interest since most of the evidence to date indicates that vitamin B_{12} in any significant amount is not present in the leaves of higher plants.

In a more recent publication, these workers have attempted to characterize more completely the vitamin B_{12} activity of turnip greens grown at Blairsville, Georgia (Gray and L. J. Daniel, *J. Nutrition* **67,** *623 (1959)*). Crops grown at Blairsville and at Experiment were compared in a basal corn-soybean type diet low in vitamin B_{12} which was fed to chicks. The results indicated clearly that Blairsville turnip greens contained vitamin B_{12} activity while the crop grown from the same seed stock at Experiment did not. Growth responses to crystalline vitamin B_{12} were similar to those obtained by the Blairsville turnip greens. Furthermore, the vitamin B_{12} content of the livers of chicks on the Blairsville diet was twice as high as that when the turnip greens from Experiment were fed.

All evidence thus pointed toward the presence of vitamin B_{12} in the turnip greens grown at Blairsville, Georgia, and the absence of the vitamin in the same crop grown at Experiment.

The identity of the vitamin B_{12}-active substance was investigated using electrophoresis and chromatography. By these criteria, there was no evidence that the substance in the Blairsville turnip greens differed from cyanocobalamin.

The authors recognize that all evidence to date supports the view that original sources of vitamin B_{12} are confined to microörganisms. Since the turnip leaves were carefully washed, soil microbe contamination would not seem very likely. However, the possibility that vitamin B_{12}-producing bacteria are living epiphytically on the turnip green leaves in the Blairsville location is recognized as a possibility.

These results are of particular interest since they again emphasize that the environment in which a plant is grown can have influences measurable by nutritional studies with animals.

ESTROGENS IN PASTURE FORAGES

Many pasture plants common in temperate zones have been shown to contain estrogens, but these levels have not been sufficient to suggest that they might cause changes in milk composition.

The discovery that estrogens are present in clovers and in some grass varieties at certain times of the year has stimulated the assay of a large number of pasture plants by G. S. Pope, M. J. McNaughton and H. E. H. Jones (*J. Dairy Res.* **26,** *196 (1959)*). Their results are of particular interest since there is such a widespread use of estrogens in fattening operations with sheep and cattle, species which normally obtain a large proportion of their feed from pastures.

While all of the plants they assayed were found in British pastures, the widespread use of many of these plants in temperate zones around the world increases the application of their findings. Estrogens have become of increasing concern in the nutrition of animals since E. M. Bickoff *et al.* (*Science* **126,** *969 (1957)*) demonstrated the presence of coumestrol in *Trifolium repens*, the common ladino clover. They showed that its estrogenic potency was about 30 times greater than that of genistein or biochanin A, compounds which have been found in subterranean clover by R. B. Bradberry and D. E. White (*J. Chem. Soc.* **4,** *3447 (1951)*), a plant which under certain conditions, at least, has produced a pathological effect in breeding sheep because of its

excessive estrogen stimulation (H. W. Bennets, *J. Depart. Agric. West. Australia* **21,** *104 (1944)*).

Additional concern over the presence of estrogens in pasture plants has arisen since the demonstration by S. J. Folley (*Biochem. J.* **30,** *2262 (1936)*) and by Folley, H. M. S. Watson and A. C. Bottomley (*J. Dairy Res.* **12,** *1 (1941)*) that estrogens administered to lactating cows can cause an increase in the percentage of fat and non-fatty solids in the milk.

The studies of Pope, McNaughton and Jones with the immature mouse indicated the presence of estrogens in alfalfa, in various species related to alfalfa, in clovers and in a number of grasses. However, none of the levels was high enough to produce changes such as those described by Folley. Nevertheless, since these observations were not closely related to environmental conditions, while reassuring in themselves, they do not rule out the possibility for circumstances which may result in such high levels of estrogen as those that produced pathological effects in Australia.

THE LAXATIVE EFFECT OF DIETARY ROUGHAGE

Feeding wheat bran to pigs increased the water content of their feces. The effect was reproduced with pure cellulose fed in a fibrous form, but not with powdered cellulose.

The practice of adding wheat bran to a human diet in order to increase bowel movements and make them easier is well known. P. H. Cooper and C. Tyler (*J. Agr. Sci.* **52,** *332, 340 (1959)*) have reported a recent study of the mechanism for this effect, using the pig whose digestive system has some similarities to that of man.

In the first experiment two newly weaned pigs were used. For three weeks pig A received two daily feeds of 750 g. dry meal and 1750 ml. water, with the meal made up of 60 parts ground maize, 30 parts ground barley, 10 parts fish meal and 1 part salt. Pig B received a similar treatment with 25 parts of wheat bran and 10 parts of starch introduced into its meal of maize. This change increased the cellulose content (as determined by the procedure of L. E. Wise, M. Murphy and A. A. D'Addieco, *Paper Trade J.* **122,** *35 (1946)*) from 4.3 per cent of the dry meal to 9.2 per cent, and the crude fiber content from 2.4 to 4.8 per cent.

In a 24-hour period pig A passed six stools with a total weight of 700 g., while pig B passed ten stools with a weight of 1400 g. Much of this difference in weight consisted of water, while the percentage of dry matter in the two series of stools averaged 29.6 and 19.6 respectively. The 24-hour output of dry matter was 206 g. for pig A and 277 g. for B, and included 40 and 71 g., respectively, of undigested cellulose.

At the end of the experiment the pigs were killed four hours after their morning feed, and the digestive tract was rapidly tied off into sections. The total dry matter in the tract was very similar for the two pigs, being 640 g. for pig A and 680 g. for pig B. However, there were large differences in the total moisture content, with values of 2130 and 3170 g. respectively. The greatest differences were found in the cecum, colon and rectum, while there was less difference in moisture content in the earlier portions of the tract.

The second experiment was designed to test whether the laxative effect of the bran could be reproduced by adding cellulose alone to the same basal diet. The first preparation was in a finely powdered form containing 80 per cent cellulose and 20 per cent hemicellulose. The amount fed to each pig was gradually increased to 25 per cent for one and 10 per cent for the other. However, this powdered cellulose preparation had no laxative effect, although large weights of dry matter were excreted. The stools were in the form of hard pellets and their mean

dry matter contents were 38 and 43 per cent, respectively.

The diets were then changed. The first pig was transferred to a fibrous cellulose preparation, fed at the 5 per cent level. This consisted of filter paper cake (without asbestos) obtained in block form and macerated with water before being mixed with the dry food. Analysis showed it to be pure cellulose. For the second pig the powdered cellulose was still used but the level was reduced to 5 per cent.

There was a considerable difference in the excretions of the two pigs. The stools of pig C receiving the fibrous cellulose contained 24 per cent of dry matter, while those of pig D receiving the powdered material were considerably drier, containing 34 percent dry matter. Pig C also excreted a greater weight of dry matter so that the 24-hour wet weight of its stools was 1250 g. as compared to only 740 g., for pig D. *In vitro* tests with the two cellulose preparations also demonstrated that the powdered preparation absorbed and retained much less water than the fibrous one.

In further experiments, the fibrous cellulose was added (at the expense of starch) to a purified diet made up of 50 parts dried skim milk, 44 parts starch, 5 parts dried brewers yeast and 1 part salt. Adding 15 and 25 per cent of fibrous cellulose resulted in extremely wet stools with only 17 and 15 per cent, respectively, of dry matter as compared with the values of 26 to 34 per cent when little or no cellulose was added to the same basal diet. This meant that when the 25 per cent level of cellulose was fed, 1660 g. of water were excreted per 24 hours in the stools, but only 300 g. in the urine.

The authors also report the time pattern of excretion throughout the 24 hours in each experiment. They conclude that, in general, excretion takes place during periods of maximum flow of gut contents from the small into the large intestine, and is not necessarily related to the actual mass of material present in the lower gut. This is in agreement with the conclusions of K. L. Blaxter, N. McC. Graham and F. W. Wainman (*Brit. J. Nutr.* **10,** *69 (1956)*) who made their observations on sheep.

Cooper and Tyler also found large differences in the dry matter content of the stools produced at different points in the 24-hour cycle. In general, those produced in the early morning (which presumably had been longest in the lower gut) were the driest, as might be expected.

The general conclusion that can be drawn from this work is that it is not the cellulose content per se of wheat bran that gives it its laxative effect. This was shown by the failure of a cellulose preparation in powdered form to reproduce the laxative effect of bran. It may be, however, that the physical condition or structure of the cellulose and other relatively indigestible polysaccharides in bran are responsible for the laxative effect because of their power to retain water. Pure cellulose in fibrous form seemed to have an effect very similar to that of bran and to illustrate this mode of laxative action.

VITAMIN B_{12} INTERRELATIONSHIPS

Dietary riboflavin and folacin appear to spare vitamin B_{12} in weanling rats on a low B_{12} diet. Choline may have the opposite effect.

Dietary vitamin B_{12} is stored by mammals primarily in the liver and kidney. R. A. Harte, B. F. Chow and L. Barrows (*J. Nutrition* **49,** *669 (1953)*) and R. F. Schilling (*Am. J. Clin. Nutrition* **3,** *45 (1955)*) have shown that the vitamin can be firmly attached to globulins and seems to be held tenaciously in emergency states. Liver storage is increased when the vitamin is increased in the diet (A. E. Schaefer, W. D.

Salmon and D. R. Strength, *Proc. Soc. Exp. Biol. Med.* **71,** *193 (1949)*).

L. N. Ellis, B. J. Duncan and I. B. Snow (*J. Nutrition*, **67,** *185 (1959)*) have used the storage of vitamin B_{12} in the liver to indicate the interrelationship between vitamin B_{12} and riboflavin, folacin and choline at levels likely to occur in human diets. They measured the storage of B_{12} in the livers of male weanling rats that had been subjected to a period of low intake of the vitamin supplemented by either low or high amounts of the other three vitamins. The experimental period lasted four weeks. Food intake was equalized between the litter mates and the animals were weighed weekly.

A fivefold dietary increase of riboflavin resulted in a small increase in total vitamin B_{12} in both liver and kidney when calculated per gram of dry tissue or nitrogen. This indicates a sparing action of riboflavin at sub-optimal levels of vitamin B_{12}. The amount of vitamin B_{12} per gram of fresh tissue or per total organ was similarly increased in the liver, but a decrease was observed in the case of the kidney because of hydration and lower kidney weight.

The ratio of free to bound vitamin B_{12} in these organs increased on the high-riboflavin diet, the percentage of free vitamin B_{12} rising from 88 to 100 per cent in the liver and, insignificantly, from 79 to 81 per cent in the kidney. It is not known whether this change made the vitamins more or less available for metabolism. Apparently the mechanism for storage under these conditions did not involve protein binding. Although the nature of the free vitamin B_{12} was not clear from these studies, the vitamin B_{12} of human liver has been found to be readily available to *Euglena gracilis* (W. R. Pitney, M. F. Beard and E. J. Van Loon, *J. Biol. Chem.* **212,** *117 (1955)*).

The authors point out that, although the increase of stored vitamin B_{12} on the high-riboflavin diet could have resulted from an increased release of vitamin B_{12}-active fragments from riboflavin during digestion, an actual decrease of stored riboflavin on this diet suggests a metabolic interrelationship.

A fivefold increase of folic acid in the diet resulted in a small, statistically non-significant, increase of total vitamin B_{12} in the liver and kidney, but in this case the increase occurred primarily in the bound form. Furthermore, increasing the folic acid of the diet resulted in significant increases of hemoglobin and of stored folic acid.

In contrast to the results with riboflavin and folic acid, a twofold increase of choline in the diet caused a significant decrease of total and free vitamin B_{12} in both liver and kidney to approximately the level found with a very deficient diet. The decreased storage occurred with little or no change in the hemoglobin level or in the storage of choline in the liver and kidney.

The authors are of the opinion that, although the decreased storage of vitamin B_{12} may have resulted from renal lesions and fatty infiltration of the organs during the two weeks depletion period on a choline-free diet, the negligible decrease in nitrogen content contradicts such changes as being major factors. Moreover, animals on a low-choline diet were able to deposit vitamin B_{12}, when added to the diet, at a level comparable to that found in animals on either the low-riboflavin or low-folic acid diets. The authors conclude that the present studies provide no material for interpretation.

When a comparison of results from each of the three series is made, it is seen that at each dietary level of vitamin B_{12} the total vitamin B_{12} calculated per gram of body weight was highest for the folic acid diets. The lowest value of vitamin B_{12}, on a fresh weight basis, was 0.037 μg. per g. of fresh liver for the diet deficient in both vitamin B_{12} and choline. This value is higher than that reported by H. E. Scheid, M. M. Andrews and B. S. Schweigert (*Proc. Soc. Exp. Biol. Med.*) **78,** *558 (1951)*) (0.026 μg.), as well as that reported by M. J. Burns and Salmon (*J. Agr. Food Chem.* **4,** *257 (1956)*) (0.005 μg.).

POTASSIUM-LYSINE RELATIONSHIPS

Increasing the potassium content of a lysine-deficient diet appears to compensate partially for the deficiency.

It has been demonstrated by R. E. Eckel, C. E. Pope II and J. E. C. Norris (*Arch. Biochem. Biophys.* **52,** *293* (*1954*)) and M. Iacobillis, E. Muntwyler and C. L. Dodgen (*Am. J. Physiol.* **185,** *275* (*1956*)) that in the rat a loss in muscle potassium is at least partially compensated by a gain in free lysine, which acts as a cation. This fact suggested to S. N. Gershoff *et al.* (*J. Nutrition* **67,** *29* (*1959*)) that a converse compensation might occur. The effect of altering potassium levels in diets containing varying quantities of lysine was therefore studied.

Groups of eight male weanling Charles River CD (Caesarian delivery) rats, housed in group cages, were used for this study. The diets were composed primarily (89.5 per cent) of a commercial dry breakfast cereal containing 3.12 per cent nitrogen (mostly from rice and wheat gluten and from small amounts of skim milk). The available lysine was found to be only 0.31 per cent. When this diet was supplemented with lysine, rat growth equal to that obtained with isonitrogenous casein-containing diets was achieved.

Other components of the basic diet included 4.0 per cent corn oil, 1.0 per cent cod liver oil, 0.3 per cent choline and adequate vitamin supplements. Diets two, four and six contained 4 per cent Salts IV, and diets one, three and five contained 1.2 per cent disodium phosphate and 2.8 per cent Salts IV minus its potassium salts.

Analysis for lysine and potassium showed that diet one contained 0.28 per cent lysine and 0.14 per cent potassium. Diet two contained 0.28 per cent lysine and 0.72 per cent potassium. Diet three contained 0.53 per cent lysine and 0.14 per cent potassium. Diet four contained 0.53 per cent lysine and 0.72 per cent potassium. Diet five contained 1.28 per cent lysine and 0.14 per cent potassium. Diet six contained 1.28 per cent lysine and 0.72 per cent potassium. Sodium content of the odd numbered diets was 1.27 per cent and for the even numbered ones, 0.96 per cent. The diet and water were given ad libitum.

After six weeks, the animals were killed and samples of liver, shaved skin and muscle were obtained for analyses of potassium and sodium, nitrogen and amino acids.

Differences in growth were related to the lysine but not to the potassium content of the diets. During the third week, the rats in group one started losing hair and by the end of the experiment showed marked alopecia. Although some of the rats in group two lost some hair during the six weeks, the least involved rat in group one lost considerably more hair than the most involved animal in group two. This, the authors suggested, was clearly a protective effect of increased potassium in diets deficient in lysine.

Histological changes most suggestive of a protective effect of excess dietary potassium in the presence of a lysine deficiency included the liver, in which the amount of sudanophylic material could be correlated with the dietary lysine levels. The livers of rats from groups one and two had moderate amounts of lipid and those of groups three and four had minimal amounts, while those of the two highest lysine groups showed only the minute traces of lipid seen in livers of normal rats. A potassium effect was apparent only at the 0.28 per cent lysine level, where it was associated with a reduction in the amount of liver lipid.

The thickness of the epiphyseal cartilage of the distal end of the radius was measured with the aid of a calibrated ocular grid. At the lowest lysine level, a distinct thinning of the epiphyseal plate could be seen. At

each level of lysine, it was observed that increasing the potassium level of the diet was accompanied by an increased thickness of the epiphyseal plates. However, not all differences were statistically significant.

Various other histological changes could not be related to dietary potassium levels. At the various lysine levels fed, the potassium content of the diets had no significant effect on the tissue concentrations of nitrogen, sodium or potassium. The lysine content of the diet did not affect the sodium and potassium concentrations of muscle and liver, but markedly increased the sodium and potassium content of the skin. There also appeared to be a slight effect of high lysine diets in increasing the nitrogen concentrations of the tissues examined.

Differences in the amino acid paper chromatographic patterns of the various dietary groups were not marked, but a decrease in dietary lysine was associated with increases in muscle lysine and histidine and in the spot representing liver phenylalanine, leucine and isoleucine. In those cases in which phenylalanine could be separated from leucine and isoleucine, it was observed that the changes had actually taken place in the leucine-isoleucine component of the spot. A decrease in the density of the spot representing liver glutamic acid and threonine was also associated with lysine deficiency. The amino acid changes could not be correlated to any detectable degree to changes in the potassium content of the diet.

Because of its abundance in plant and animal tissues, potassium is usually not given consideration as a dietary adjunct in practical nutrition. The authors point out, out, however, that although primary potassium deficiency in humans may not exist commonly, problems involving potassium nutrition may occur as a result of disease or aberrant food supplies. J. D. L. Hansen and J. F. Brock (*Lancet* **II,** *477 (1954)*) found that infants suffering from nutritional edema (some with kwashiorkor) had an increased need for potassium.

Moreover, potassium requirements seem to increase when tissue proteins are in process of repletion. The need for potassium in post-operative surgical cases as a protection against nitrogen loss has been stressed by L. P. Eliel, O. H. Pearson and F. C. White (*J. Clin. Invest.* **31,** *419 (1952)*) and by D. V. Frost and H. R. Sandy (*Proc. Soc. Exp. Biol. Med.* **83,** *102 (1953)*). The increased need for potassium by rapidly growing guinea pigs has been pointed out by P. Roine *et al.* (*Ibid.* **71,** *90 (1949)*) and H. R. Heinicke, A. E. Harper and C. A. Elvehjem (*J. Nutrition* **58,** *269 (1956)*).

FACTORS CONTROLLING CHOLESTEROL EXCRETION

In the hypercholesteremic rat, 4 per cent taurine reduced serum, liver, and aorta cholesterol significantly. In the hypercholesteremic rabbit, 2 per cent beta sitosterol reduced serum, liver, and aorta cholesterol but taurine and glycine had no significant effect.

Experiments using C^{14}-cholesterol have demonstrated that cholesterol is eliminated mainly by biliary excretion of bile acids (M. D. Siperstein, F. M. Harold, I. L. Chaikoff and W. G. Dauben, *J. Biol. Chem.* **210,** *181 (1954)*). G. A. D. Haslewood (*Physiol. Rev.* **35,** *178 (1955)*) and J. Bremer (*Biochem. J.* **63,** *507 (1956)*) point out that bile acids are conjugated with taurine in the rat, with glycine in the rabbit and with either taurine or glycine in man or the monkey. The combination of diets high in cholesterol and deficient in sulfur-containing amino acids produces hypercholesteremia in the Cebus monkey, rat and mouse according to the report of G. V. Mann, S. B.

Andrus, A. McNally and F. J. Stare (*J. Exp. Med.* **98,** *195* (*1953*)) and L. C. Fillios and G. V. Mann (*Metabolism* **3,** *16* (*1954*)). The restoration of cystine or methionine, however, effected a significant reduction in the hypercholesteremia. Some cystine is converted to taurine in mammals (L. Eldjarn, *J. Biol. Chem.* **206,** *483* (*1954*)) and S^{35}-labeled taurine is utilized for conjugation with bile acids (O. W. Portman and G. V. Mann, *Ibid.* **213,** *733* (*1955*)).

These results stimulated R. G. Herrmann (*Circ. Res.* **7,** *224* (*1959*)) to study the effect of taurine on hypercholesteremia resulting from cholesterol feeding. The effects of glycine and beta sitosterol were also studied.

The experimental animals were female rabbits weighing about 1.5 kg. and female albino rats weighing about 200 g. The animals were caged separately and food consumption was recorded. For the rabbits, commercial rabbit pellets were sprayed with the cholesterol and other supplements in an appropriate solution, while the rat diets consisted of pulverized chow mixed with the supplement in a mechanical mixer.

All animals were weighed weekly, and blood samples were taken for cholesterol determination. When the animals were killed at the end of the experiment, their tissues were examined both grossly and microscopically. Liver samples and entire aortas were frozen until cholesterol analyses could be performed.

In the rat experiment, all animals received a preliminary feeding for three weeks of a chow diet containing 2 per cent cholesterol and 1 per cent cholic acid. Group one was continued on the diet, while group two received the original diet, except that 4 per cent of taurine replaced an equal amount of chow. The diet for group three contained 4 per cent of glycine instead of taurine.

The serum cholesterol averaged 72 mg. per cent initially. However, during the first three weeks on the diet all serum cholesterol levels increased. Group one averaged 249 mg. per cent, group two averaged 227 mg. per cent and group three averaged 307 mg. per cent. The group continuing the original diet showed a further increase in serum cholesterol to 312 mg. per cent during the fourth to seventh week. On the other hand, the average serum cholesterol level in taurine supplemented rats dropped to 202 mg. per cent while the glycine group with an average serum cholesterol level of 298 mg. per cent was essentially unchanged. In the period from eight to 11 weeks group one remained unchanged, group two showed a further drop to 180 mg. per cent and group three showed a small decrease to 279 mg. per cent.

Liver cholesterol averaged 6400 mg. per cent for group one with no taurine but was 2260 mg. per cent for group two supplemented with taurine (225 mg. per cent in normal rats). Similarly, the aorta cholesterol averaged 524 mg. per cent without taurine, but was 364 mg. per cent with taurine (152 mg. per cent in normal rats). No evidence of toxicity was seen either grossly or by microscopic examination of the tissues.

In the rabbit experiment, the preliminary diet was 96 per cent rabbit pellets, 1 per cent cholesterol, and 3 per cent cottonseed oil. After four weeks on this diet, four groups were formed of seven rabbits each. The preliminary diet was continued for group one, while for the other groups 2 per cent supplements of either taurine, glycine or beta sitosterol replaced an equal weight of rabbit pellets. These diets were fed for nine weeks, at which time the animals were killed for tissue cholesterol determination and pathological examination.

The initial serum cholesterol concentrations ranged from 47 to 55 mg. per cent, while four weeks after the preliminary diet was fed the average serum cholesterol levels ranged from 186 to 278 mg. per cent. After four weeks on the various experimental diets the serum cholesterol concentrations continued to increase, except in the group supplemented with beta sitosterol. The serum cholesterol in this group averaged

only 184 mg. per cent while the other values were 331 mg. per cent for the taurine group, 350 mg. per cent for the glycine group, and 374 mg. per cent for the control group one. After 13 weeks, the beta sitosterol supplement resulted in a cholesterol level of 233 mg. per cent, while the controls averaged 531 mg. per cent, the taurine group averaged 600 mg. per cent and the glycine group was 710 mg. per cent.

Thus, although variation was great among individual animals and from time to time, nevertheless the trends indicated no effect from taurine or glycine but a significant depression of cholesterol by beta sitosterol. This depressive effect was mirrored in cholesterol values of liver and aorta.

Cholesterol concentration in normal rabbits receiving no cholesterol supplement was 260 mg. per cent for the liver and 391 mg. per cent for the aorta. The control cholesterol-fed animals, on the other hand, had liver cholesterol values averaging 710 mg. per cent and aorta cholesterol averaging 1046 mg. per cent. Neither taurine nor glycine altered appreciably the deposition of cholesterol in either the liver or aorta. However, the average liver cholesterol concentration of the beta sitosterol group was 465 mg. per cent and the average aorta concentration was 323 mg. per cent.

The most weight was gained by the control group, an average of 1243 g. in 13 weeks, while the group receiving the beta sitosterol gained the least, an average of 905 g.

Although the standard errors were very high (varying between 25 and 50 per cent, for example, in the rabbit serum cholesterol concentrations), the data indicate that serum and tissue cholesterol is lowered by beta sitosterol feeding.

The failure of either taurine or glycine to affect the cholesterol level in the rabbit is puzzling. Even if the mechanism of action of taurine in the rat is unrelated to cholic acid conjugation, a similar action might be expected in the rabbit. The results, therefore, suggest a fundamental difference in the control of cholesterol excretion between rabbits and rats. Further study of this phenomenon may reveal why rabbits fed cholesterol readily develop hypercholesterolemia and atherosclerosis while rats are much more resistant to this effect.

DIET AND CHOLESTEROLEMIA IN THE RAT

A diet promoting good growth in the rat, while leading to high serum cholesterol, contains 25 per cent saturated fat, 1 per cent cholesterol and 0.5 per cent cholic acid.

The difficulties of carrying out dietary experiments with human subjects are obvious and, therefore, expediency rather than choice dictates the use of experimental animals. The commonest laboratory animal used is the albino rat, in which many of the nutritional disorders of man may be studied in at least a preliminary manner. There are several human nutritional diseases, however, to which the rat is not very susceptible. In particular, the rat does not appear to succumb naturally to many of the common disturbances of lipid metabolism. The disease of atherosclerosis, which is so universal in civilized man, has been induced in the chick, rabbit and hamster by the use of high cholesterol diets (*Nutrition Reviews* **9,** *295 (1951)*; **10,** *148 (1952)*), while the dog and rat are more refractory to this treatment.

The assumption that the degree of atheromatosis is correlated with hypercholesterolemia has led many workers to use this latter criterion as the end result of their dietary manipulations. Hypercholesterolemia, however, is not readily achieved in the rat, nor does it appear to be correlated directly with atheromatosis (*Nutrition Reviews* **11,** *152 (1953)*; **12,** *220 (1954)*). Nevertheless, a high fat diet with additional cholesterol and bile salts has been used to achieve both hyper-

cholesterolemia and atheromatous deposits in the rat, particularly if the animals are first rendered hypothyroid either with radioiodine or with thiouracil (*Ibid.* **11,** *152 (1953)*). However, to facilitate the use of the rat as an experimental animal for this disease, more knowledge of the dietary conditions leading to hypercholesterolemia, with or without atheromatous deposition, is necessary.

Such an attempt has been reported by N. Nath, R. Wiener, A. E. Harper and C. A. Elvehjem (*J. Nutrition* **67,** *289 (1959)*) by taking into consideration such factors as dietary saturated and unsaturated fat, pyridoxine deficiency, cholesterol, cholic acid and thiouracil.

Male weanling rats were maintained on a diet consisting of 20 per cent casein (supplemented with 2 per cent DL-methionine), 4 per cent salts and adequate vitamin supplements, with the remainder consisting of sucrose and hydrogenated coconut oil.

In the first experiment, it was found that growth at five and ten weeks was greater with 10 per cent than with 1 or 25 per cent hydrogenated coconut oil. Apparently the rats were suffering from an essential fatty acid deficiency promoted by the high content of saturated fat (H. J. Deuel, Jr., *et al.*, *J. Nutrition* **55,** *337 (1955)*), since growth was normal and the dermatosis which developed was prevented when 1 per cent corn oil was included in the diet. The serum cholesterol was not affected by increasing the level of the dietary hydrogenated coconut oil. However, the addition of 1 per cent dietary cholesterol increased hypercholesterolemia about fourfold (to 336 mg. per cent), while the addition of 1 per cent corn oil in the presence of 1 per cent dietary cholesterol lowered this latter value to 209 mg. per cent. The liver lipids and liver cholesterol were greatly increased by dietary cholesterol, as would be expected.

In the second experiment, the effects of pyridoxine deficiency were studied. Very poor growth and severe dermatosis were observed after 15 weeks on a diet deficient in pyridoxine but including 25 per cent hydrogenated coconut oil and 1 per cent cholesterol. Corn oil alleviated the dermatosis, but pyridoxine was necessary to bring about normal growth. As before, serum and liver cholesterol values were elevated by dietary cholesterol.

In the third experiment, the additional effects of 1 per cent cholic acid and 0.3 per cent thiouracil were tested. When the cholic acid was added to a diet containing 25 per cent hydrogenated coconut oil and 1 per cent cholesterol, weight gain was somewhat reduced while the serum and liver cholesterols were increased two to threefold at the end of 12 weeks. Thiouracil increased the serum cholesterol an additional threefold, but actually lowered liver cholesterol and severely depressed growth.

Corn oil had some serum cholesterol-lowering effect in the presence of cholic acid, but did not influence liver cholesterol. In those diets where thiouracil was present, corn oil had no effect. It is unfortunate that the use of higher levels of corn oil was not attempted since, in most studies, little cholesterol-lowering effect is noted until the unsaturated fat becomes a sizable proportion of the total diet.

In the fourth experiment, an attempt was made to ascertain the optimum proportions of cholic acid and cholesterol for production of high serum cholesterols without undesirable side effects such as diarrhea. It was decided that pyridoxine and thiouracil should not be excluded from the diet since deficiencies of these favor anorexia and unfavorable tissue changes. Both dietary cholesterol and cholic acid induced hypercholesterolemia with the cholic acid having the greater influence. The highest serum cholesterol values were obtained when 0.75 per cent of each factor was included in the diet, but some diarrhea was present.

Nath and his co-workers therefore propose, on the basis of these studies, that an experimental diet promoting both good

growth and a significantly high serum cholesterol level (from about 80 to 450 mg. per cent in three weeks) contains 25 per cent hydrogenated coconut oil, 1 per cent cholesterol and 0.5 per cent cholic acid.

The authors state that variations in the fat content of the diet had little effect on the serum cholesterol of the rats. The high levels of hydrogenated coconut oil were used primarily in order to promote an essential fatty acid depletion since it has been speculated that the essential fatty acids may be important in regulating the serum cholesterol level (H. M. Sinclair, *Lancet* **I,** *381* (*1956*)). An explanation for the action of cholic acid is that it promotes cholesterol absorption resulting in higher levels of serum cholesterol and serum lipid phosphorus as well as deposition of cholesterol in the liver. Histological examination of the cardiovascular system was not carried out in this series of studies.

It seems likely that these observations may be valuable in the formulation of useful diets for studying serum and liver cholesterol changes in the rat. However, there are several indications that studies in the rat cannot be directly applicable to the human disease. First, the rat does not develop a significant spontaneous atherosclerosis and it is necessary to employ relatively severe dietary conditions. Second, dietary fat seems to have less influence on the serum cholesterol in the rat than in man (*Nutrition Reviews* **13,** *8*, *44*, *138* (*1955*)). Finally, dietary cholesterol appears to be effective in promoting hypercholesterolemia in the rat while there is no conclusive evidence that this sterol influences human cholesterol levels (*Nutrition Reviews* **14,** *327* (*1956*)).

Therefore, despite the many limitations for experimental study, the ultimate evaluation must be done on man, although preliminary data may be obtained with experimental animals such as the rat.

VITAMIN D AND INTESTINAL ABSORPTION OF RADIOCALCIUM

Active transport of Ca^{45} has been demonstrated using everted sacs of small intestine. Transport through sacs prepared from rats fed a vitamin D-deficient diet was depressed.

For many years it has been known that vitamin D influences the intestinal absorption of calcium (*Nutrition Reviews* **13,** *271* (*1955*); **16,** *148* (*1958*)), but relatively little is known about the mechanism of this effect. Most studies of the problem have been made using *in vivo* techniques and, although these studies have clearly shown a need for vitamin D for normal calcium absorption, they have provided little information about the nature of the interrelationships involved in the absorption process.

D. Schachter and S. M. Rosen (*Am. J. Physiol.* **196,** *357* (*1959*)) have approached the problem through *in vitro* methods, using everted sacs from segments of rabbit, rat, and guinea pig intestine. Segments of the upper small intestines of 24-hour-fasted animals were taken, washed in cold saline, cut into short lengths, and formed into closed sacs. The sacs were filled with a standard medium containing Ca^{45} and the same medium was used to bathe the outsides of the sacs. They were placed in Warburg flasks and allowed to respire for three hours at 37°C under an atmosphere of oxygen.

At the end of the incubation period the inside (serosal) and the outside (mucosal) solutions were collected and the Ca^{45} concentration of an aliquot of each solution was determined. Tissue uptake of Ca^{45} was estimated by subtracting the final Ca^{45} content of the medium from the initial Ca^{45} content of the medium. The validity of the

estimation of tissue uptake was verified by ashing the tissue in nitric acid, precipitating the calcium as the oxalate, and determining the Ca^{45} content of the precipitate.

A measure of the extent of active transport was obtained by calculating the Ca^{45} concentration ratio, I/O, between the inside and outside media. Ratios for I/O greater than 1, indicating transfer against a concentration gradient, were taken as evidence of active transport of Ca^{45}. In a large number of tests with preparations from normal animals, ratios of from 3 to 5 were obtained. The process of active transport could be shown in this system to require the simultaneous occurrence of oxidative metabolism since the replacement of oxygen by nitrogen, reduction of the incubation temperature to 5°C, or the presence of sodium fluoride depressed the I/O ratios to values not exceeding 1.0. Tissue uptake occurred, however, despite the inhibition of oxidative metabolism, possibly owing to exchange reactions of Ca^{45} with bound calcium in the tissues.

Various metabolites including amino acids, tricarboxylic acid cycle intermediates, fatty acids and carbohydrates were tested for their influence on the transport process. Only glucose, fructose, and maltose appeared to stimulate Ca^{45} uptake. Glucose and maltose increased the amount of transport to double that of the control without substrate, and fructose increased it even more. Citrate and oxalate were inhibitory.

Three groups of compounds with inhibitory action were tested. The presence of 2,4-dinitrophenol completely inhibited Ca^{45} transport, although oxygen consumption was increased by 20 per cent, suggesting that (in common with other active transport mechanisms) the transport of Ca^{45} is dependent on the production of high energy phosphate bonds. Several divalent cations were tested as possible competitive inhibitors of calcium transport. Magnesium and cobalt ions, in contrast to strontium and barium ions, produced a consistent depression in the amount of Ca^{45} transport. The sodium salts of versene, glucuronic acid, and citric acid, all chelating agents for calcium, inhibited the transport of Ca^{45}.

Intestinal segments from rabbits and rats maintained on a rachitogenic diet were less effective in transporting Ca^{45} than were similar segments from animals maintained on the same diet but supplemented with vitamin D. A depression in Ca^{45} transport could be demonstrated in intestinal segments from rats within two days after the animals had been given a rachitogenic diet.

This stimulated the authors to examine the possibility that the inhibition of citrate oxidation by vitamin D (H. F. De Luca *et al., J. Biol. Chem.* **224,** *201 (1956)*) and the consequent accumulation of a calcium-citrate complex in the serosal fluid might be essential for the active transport of Ca^{45} and for the effect of vitamin D on calcium transport. From a knowledge of the citrate concentration in the medium and as a result of calculations based on the pK value for the calcium citrate complex, the authors concluded that active transport of calcium cannot be mediated through a calcium-citrate complex and that the effect of vitamin D on citrate oxidation cannot be responsible for its effect on calcium absorption.

Although the information obtained by the authors provides evidence that calcium is actively transported by the small intestine through a mechanism that is dependent on oxidative phosphorylation and dietary vitamin D, the importance of active transport of calcium *in vivo* cannot be evaluated from the results of these *in vitro* experiments. Since the active transport mechanism can transport calcium against a calcium concentration similar to that in plasma, the authors conclude that the participation of active transport *in vivo* is possible. Transport by passive diffusion in the intact animal, however, could occur if there were a calcium concentration

gradient from the lumen of the small intestine to the bloodstream.

Also the experiments do not provide evidence about the way in which vitamin D influences calcium absorption. It is of considerable interest, however, that a fall in the extent of active transport of calcium could be detected using sacs that were prepared from rats fed a vitamin D-deficient diet. The activity could not be restored by adding calciferol in propylene glycol directly to the medium. This suggests, but is not of course conclusive evidence, that the effect of vitamin D is indirect. The possibility that an *in vitro* system in which vitamin D is active might be obtained by some method of preincubating the sacs from rachitic animals with vitamin D would appear to be worthy of further investigation.

TOCOPHEROL DERIVATIVES IN EXPERIMENTAL DYSTROPHY

Alpha-tocopherol, its acetate, hydroquinone and hydroquinone disuccinate cure experimental dystrophy if administered orally. Tocopherol and the hydroquinone are effective intravenously but the esters are not.

The relationships between alpha-tocopherol and its derivatives and muscular dystrophy in man and experimental animals are not clear (*Nutrition Reviews* **3,** *259* (*1945*); **4,** *324* (*1946*)). Since in the latter, the disease can be produced by vitamin E-deficient diets, often with peroxidized oil, it should be possible to delineate exactly the conditions under which various tocopherol derivatives will present or alleviate the typical symptoms such as creatinuria and observable muscular lesions.

However, although oral administration of alpha-tocopherol was shown to cure experimental dystrophy in rabbits and rats and the tocopherol hydroquinone was found to be effective when given intravenously, the hydroquinone appeared to have much less effect when given orally to hamsters (W. T. West and K. E. Mason, *Am. J. Phys. Med.* **34,** *223* (*1955*)), and no effect when fed to children (P. L. Harris and K. E. Mason, *Am. J. Clin. Nutrition* **4,** *402* (*1956*)). Esters of the hydroquinone, which should be readily hydrolyzed in the body, also appeared to be ineffective.

In order to clarify these observations, J. B. Mackenzie and C. G. Mackenzie (*J. Nutrition* **67,** *223* (*1959*)) fed young rats a vitamin E-deficient diet containing 10 per cent lard which had been heated to destroy antioxidants. After 20 weeks on the diet, the experimental rats had gained 20 per cent less weight than the controls with about seven times the creatine excretion and some muscular lesions. Although single 5 mg. oral doses of alpha-tocopherol acetate reduced the creatinuria within three days and muscles became normal at the end of two weeks (after a total of 20 mg. of the acetate), the disuccinate of alpha-tocopherol hydroquinone had little effect, even after a two-week period of daily intravenous injections.

When dystrophic rats were given daily injections of either alpha-tocopherol or alpha-tocopherol acetate, it was found that the acetate was much slower in effecting a diminution of creatinuria or muscle lesions even though it had been equally effective when given orally. Under the same conditions, the hydroquinone appeared to be as effective as the alpha-tocopherol. However, the hydroquinone disuccinate, injected at four times the molar level of the tocopherol, was almost without effect, although it was as effective as alpha-tocopherol or the hydroquinone when given orally to dystrophic rabbits.

Thus it appears that both alpha-tocopherol and the hydroquinone can cure experimental dystrophy when given by injection.

The esters of these compounds, on the other hand, although effective by mouth, are apparently hydrolyzed too slowly in the blood to be very effective on injection. The authors point out that either the free alpha-tocopherol or the hydroquinone might be used in clinical cases in which a rapid raising of the blood level is desired.

EFFECTS OF THIOURACIL AND SITOSTEROL ON DIET-INDUCED HYPERCHOLESTEROLEMIA AND LIPOMATOUS ARTERIAL LESIONS IN THE RAT

Atherosclerotic lesions were induced in rats fed a semisynthetic diet plus thiouracil. Addition of sitosterol to this regimen protected most rats. Sitosterol interfered with absorption of cholesterol and perhaps of thiouracil.

Although the rat is quite resistant to the development of hypercholesterolemic atherosclerosis, recently devised techniques have enabled investigators to produce this condition in them (*Nutrition Reviews* **11,** *152 (1953)*; **13,** *20 (1955)*; **15,** *141 (1957)*; and W. S. Hartroff and W. A. Thomas, *J. Am. Med. Assn.* **164,** *1899 (1957)*). A combination of an atherogenic diet containing large amounts of saturated fatty acids and cholesterol together with thiouracil would produce arterial lesions similar to the human atherosclerotic lesion.

Sitosterol has been reported to protect hypothyroid rats from these atherogenic changes (M. M. ⁻st and C. H. Duncan, *Circulation* **14,** *344 (1956)*).

Best and Duncan (*Am. Heart J.* **58,** *214 (1959)*) studied the effects of both thiouracil and sitosterol upon rats fed the same atherogenic diet. Twenty-four male rats were divided into four groups of six each. The first group was fed laboratory chow and the second was fed the semisynthetic diet. Group three was fed this diet plus thiouracil. Group four was fed the diet plus thiouracil and sitosterol. These regimens were continued for fifteen weeks and then the animals were killed. Measurements were made of their serum cholesterol, hepatic cholesterol, hepatic weight, thyroid weight, and of the microscopic changes in their hearts and kidneys.

The results showed that the first two groups of animals ate well and gained weight, but groups three and four ate poorly and did not gain after the first few weeks. The serum cholesterol was lowest in the control group, higher in the group fed a special diet, extremely high in the group fed this diet plus thiouracil, and somewhat lower in the group fed sitosterol. The hepatic concentration of cholesterol, however, was highest in group two, lower in group three and still lower in group four. Hepatic weight reflected this storage of cholesterol. The thyroid glands were grossly enlarged in the group fed thiouracil, less so in the group fed sitosterol and thiouracil.

Histologic sections of heart and kidneys were stained for fats and disclosed deposits in none of group one, one heart of group two, three hearts and four kidneys of group three, and only two hearts of group four. In three rats of group three, renal infarcts were found.

The authors pointed out that sitosterol behaves in a fashion similar to cholesterol, and hence might give falsely elevated values for serum cholesterol. Also they questioned whether sitosterol might interfere with gastrointestinal absorption of thiouracil, since the thyroids of animals so treated were not as enlarged as those from animals fed thiouracil alone.

They wisely considered their groups of animals to be too small for firm conclusions. However, it appeared that hypercholesterolemic atherosclerosis occurred less frequently in rats fed sitosterol along with an

atherosclerotic ration than in those fed this ration alone. The mechanism of this effect could be interference with absorption of cholesterol or interference with absorption of thiouracil, or both.

In this preliminary report, the authors have given further evidence for dietary and hormonal regulation of the metabolism of cholesterol, and secondarily, for the occurrence of atherosclerosis. It would seem advisable to determine whether sitosterol exerts a similar effect in thyroidectomized rats fed an atherogenic diet. Also pair feeding should be considered, since the first two groups gained weight while the second two did not.

CHOLESTEROL ABSORPTION IN THE RAT

Bile salts are generally considered to facilitate the absorption of cholesterol, but the observation that bile salts inhibit cholesterol absorption in vitro suggests that these compounds may exert other effects.

The presence of bile acids in the intestine leads to higher concentrations of cholesterol in lymph, liver and blood, an effect that is generally attributed to increased intestinal absorption of cholesterol. It has been suggested that pancreatic cholesterol esterase and fatty acids are also involved in cholesterol absorption (*Nutrition Reviews* **13,** *246 (1955)*), but neither the exact mechanism of cholesterol absorption nor the specific role of bile acids in relation to the effects of these other factors is clearly understood.

In a study of the role of bile acids in cholesterol absorption, *in vitro* experiments on cholesterol uptake by inverted (more correctly everted) sacs of rat intestine have been carried out by A. L. Smith, R. Hauk and C. R. Treadwell (*Am. J. Physiol.* **193,** *34 (1958)*). They used small intestines from fasted male rats, weighing 200 to 300 g. The sacs from one intestine were randomly distributed among control and experimental groups. The basic external medium was a 0.154 molar phosphate buffer (pH 6.2) and contained per 4 ml., 0.0205 micromoles potassium chloride, 0.0154 micromoles calcium chloride, 0.0051 micromoles manganese sulfate, 120 mg. glucose and 20 mg. of purified human or bovine serum albumin. Cholesterol, oleic acid and sodium taurocholate were incorporated into the external medium by homogenization. The internal (serosal) medium was 0.5 to 1.0 ml. of the basic external medium prepared without glucose. The sacs were incubated in 4 ml. of external medium in a Dubnoff shaker under either 100 per cent oxygen or 95 per cent oxygen and 5 per cent carbon dioxide.

The incubation of such sacs in emulsions of cholesterol, either free or esterified, led to the disappearance of cholesterol from the external medium and an accumulation of free or esterified cholesterol in the sacs and against a concentration gradient. The total cholesterol content of the sacs before incubation was 1.8 to 2.4 mg. per g. while the external medium contained 1 mg. per ml. After incubation the total cholesterol content of the sacs was 4.8 to 5.4 mg. per g.

The uptake of cholesterol by sacs from various segments of the intestine from the lower duodenum to the upper ileum was constant and was independent of the pH of the external medium over the range from 5.9 to 7.2. Bile salts added to the external medium containing cholesterol inhibited cholesterol uptake, the inhibition being 97 per cent when the molar concentration of taurocholate was four times that of cholesterol. The addition of oleic acid to the medium containing cholesterol also decreased cholesterol uptake. The inhibition was less in the presence of both bile salt and oleic acid. Addition of an extract from

rat pancreas to the external medium also depressed the uptake of cholesterol.

In these studies, large variations were observed among different animals and the results were primarily of a qualitative nature. The variability was reduced in later experiments (Smith and Treadwell, *Am. J. Physiol.* **195,** *773 (1958)*) in which some of the factors involved in cholesterol uptake by inverted intestinal sacs were studied quantitatively. The conditions suggested for getting reproducible results are the use of (a) 200 to 250 g. male rats, (b) animals fasted 24 hours, (c) 3.0 cm. sacs, (d) a one hour incubation, (e) sacs not rinsed with saline after incubation.

Employing these sacs, the authors showed that glucose is not required in the medium for cholesterol uptake. The uptake of cholesterol (10 micromoles in the external medium) was 5.5 micromoles in the absence of glucose in the medium. The uptake of cholesterol when the medium contained 0.3 or 3 per cent of glucose was 6 micromoles. Results obtained with sacs incubated in a medium containing 10 micromoles of cholesterol and 0 to 120 mg. of albumin showed that albumin is not required for cholesterol absorption, but that albumin does facilitate cholesterol uptake.

Further, the presence of bile acids in the external medium was not obligatory for cholesterol uptake by the sacs. In fact, both conjugated and unconjugated bile acids inhibited cholesterol uptake, marked inhibitions being obtained with cholic and dehydrocholic acids, taurocholate and glycocholate. Purified samples of taurocholate and glycocholate were less inhibitory than commercial samples, suggesting that the latter may contain an inhibitor.

To study the mechanism of this inhibition, sacs were preincubated in an external medium containing 20 micromoles of taurocholate for various lengths of time or with different concentrations (0 to 80 micromoles) of taurocholate for one hour, and later with the medium containing cholesterol for one hour. As the taurocholate concentration of the pre-incubation medium was increased, the cholesterol uptake decreased. Cholesterol uptake also decreased as the period of pre-incubation with taurocholate increased.

These findings, according to the authors, suggest that the inhibitory effect of taurocholate is in the sac rather than in the medium, and that taurocholate is taken up slowly in the sac where it inhibits cholesterol uptake. Also, they pre-incubated sacs with taurocholate and then incubated them in media containing different concentrations of cholesterol and concluded that taurocholate inhibits cholesterol uptake nonreversibly.

Some of these observations are not in agreement with the results of other studies on whole animals in which lymph-cholesterol concentration was taken as a measure of cholesterol absorption. Thus G. V. Vahouny and Treadwell (*Am. J. Physiol.* **191,** *179 (1957)*) reported that administration of albumin in emulsions containing cholesterol, oleic acid and taurocholate to lymph-fistula rats lowered the amount of cholesterol found in the lymph in a 24 hour period.

Also, Vahouny, C. H. Woo and Treadwell (*Am. J. Physiol.* **193,** *41 (1958)*) found that in lymph-fistula animals a molar relationship of oleic acid, taurocholate and cholesterol of 8 to 4 to 1 was most effective for lipid and cholesterol absorption from the intestine. They also observed that in rats with bile fistulas and lymph fistulas, lipid and cholesterol absorption was low, and an intragastric administration of taurocholate along with cholesterol and fatty acid failed to cause any appreciable elevation of lymph-cholesterol concentration. These results suggested that a continuous supply of bile and the presence of bile acids, fatty acids and cholesterol is necessary for optimal cholesterol absorption.

The presence of bile acids or fatty acids is not obligatory for cholesterol uptake from external medium by inverted intestinal

sacs. In fact, these substances were shown to inhibit cholesterol uptake. This evidence, according to the authors Smith and Treadwell (*Am. J. Physiol.* **195,** *773 (1958)*) suggests that the solubilizing effect of bile acids is not required for cholesterol entrance into mucosal cells. They have suggested that bile acids may be required by the mucosal cells for the formation of cholesterol esters prior to the entrance of esters into the lacteals or that they may play some part in the formation of chylomicrons.

A. Pihl (*Acta Physiol. Scandinav.* **34,** *206 (1955)*) has demonstrated that bile acids at a level of 0.5 per cent in the diet have little effect on the rate of cholesterol absorption, and has suggested that a reduced rate of cholesterol catabolism combined with a normal rate of cholesterol absorption would account for cholesterol accumulation in the liver in the presence of dietary bile acids. W. T. Beher and G. D. Baker (*Proc. Soc. Exp. Biol. Med.* **98,** *892 (1958)*) have presented evidence to show that dietary cholic acid retards the rate of mobilization of liver and serum cholesterol.

Since glucose is not required in the incubation medium for the uptake of cholesterol by inverted intestinal sacs, active transport of cholesterol must require a minimum expenditure of energy. Smith *et al.* (*loc. cit.*) explain that this may be possible because of the binding of cholesterol to a cellular protein immediately after entrance into the epithelial cells as previously suggested by J. Glover and C. Green (*Biochemical Problems of Lipids, p. 359. Edited by G. Popjak and E. Lebreton. Interscience, London, 1956*).

The facilitation of cholesterol uptake by the presence of albumin was taken to suggest that albumin may facilitate cholesterol transfer to the acceptor protein on the cell walls. The possibility of acceptor protein was suggested by the separation of a cholesterol protein complex from the washings of the intestine. They proposed that the entrance of cholesterol into the epithelial cells of the mucosa is mediated by a series of transfers of cholesterol from one protein to another. The inhibition of cholesterol absorption by bile acids, according to Smith *et al.*, could be due to nonreversible binding of bile acids at the active sites of these proteins.

The results of these studies emphasize the gaps in our knowledge of the mechanism of cholesterol absorption and the need for further work on this subject.

PROTEIN, ENERGY AND POTASSIUM

Increases in both protein content and energy content of the feed increase the requirements of chicks for potassium. With increasing age, the chick's requirement for potassium is lessened.

Several investigators (B. Ben Dor, *Proc. Soc. Exp. Biol. Med.* **46,** *341 (1941)*; M. B. Gillis, *J. Nutrition* **36,** *351 (1948)*; **42,** *45 (1950)*; C. H. Burns, W. W. Cravens and P. H. Phillips, *Ibid.* **50,** *317 (1953)*) have demonstrated the necessity for including potassium in the diet of young chicks. M. B. Gillis (*loc. cit.*) found that a higher quantity of potassium was required when the phosphorus content of the diet was borderline or suboptimal, and that potassium is required for proper bone formation. Burns, Cravens and Phillips (*loc. cit.*) concluded that the requirement was in part dependent upon the growth rate of the chicks. Sodium and potassium were found to be toxic if one of these minerals was fed greatly in excess of the other.

Results obtained by R. M. Leach, Jr., R. Dam, T. R. Zeigler and L. C. Norris (*J. Nutrition* **68,** *89 (1959)*) have further suggested an increased need for potassium

when a high-protein or high-energy diet is fed.

The composition of the basal diet employed by these workers was developed by R. Dam *et al.* (*Poultry Sci.*, **36,** *1110* (*1957*)) for use in studies of unidentified chick factors. It consisted of 45.1 per cent glucose, 10.0 per cent corn oil, 33.3 per cent purified soybean protein, 3.23 per cent cellulose, 0.84 per cent DL-methionine, 0.36 per cent glycine, 5.84 per cent salt mixture, 1.25 per cent vitamin mixture, and 0.02 per cent butylated hydroxytoluene. Duplicate lots of 13 to 15 one-day-old Vantress X White Plymouth Rock male chicks were used in all experiments.

Preliminary results indicated that the basal diet was borderline in mineral content. Three experiments were, therefore, conducted in which the effect of increasing the sodium and potassium content was ascertained. Increasing the sodium from 0.26 to 0.33 per cent and the potassium from 0.27 to 0.50 per cent resulted in consistent increases in growth rate. The mineral responsible for this increase seemed to be potassium. When graded levels of potassium were fed, the results indicated that the potassium requirement for optimum growth was approximately 0.30 per cent, while 0.20 per cent was required to prevent excessive mortality.

The potassium requirement of young chicks on the basal diet was determined to be somewhat more than 0.27 per cent (0.27 per cent was the actual potassium content of the basal diet), and experiments were carried out to study the effect of dietary factors such as protein and energy content on this potassium requirement. In one experiment, diets containing 25, 30 and 37 per cent protein were fed. The diets were kept isocaloric at approximately 3.68 calories per gram by varying the fat content. When the level of potassium was inadequate, increasing the protein content from 25 to 37 per cent of the diet markedly depressed growth rate and increased mortality. However, increasing the protein level had no effect on the chicks which received sufficient potassium to achieve an optimum growth rate.

P. R. Cannon, L. E. Frazier and R. H. Hughes (*Metabolism* **1,** *49* (*1952*)) have reported that potassium is necessary for maintenance of nitrogen balance in rats. M. Iacobellis, E. Muntwyler and C. L. Dodgen (*Am. J. Physiol.* **185,** *275* (*1956*)) have shown that potassium is involved in the maintenance of certain free amino acids in cellular tissue. These results all indicate a function of potassium in protein metabolism, especially catabolism, since the higher levels of protein are probably in excess of the animals' needs.

In another experiment, diets containing from 3.35 to 4.25 calories of metabolizable energy per gram were fed. Potassium levels varying from 0.15 to 0.45 per cent were included in the 3 per cent corn oil diet, while the graded levels in the 10 and 20 per cent corn oil diets were increased according to increases in the energy content of the diet. This resulted in a constant ratio of potassium to energy for each potassium level. The results of this experiment indicated that the potassium requirement is related to the energy content of the diet rather than to the fat content per se. In the diets in which potassium was the limiting factor, the grams of gain per gram of potassium was markedly uniform at each level of potassium regardless of the fat content of the diet.

To further confirm this observation, an analysis of variance showed that highly significant growth increases ($P < 0.01$) were obtained with all diets by increasing the potassium content, but no interaction was revealed between the level of fat and the level of potassium. An improvement in growth was also obtained by increasing the fat content from 3 to 10 per cent. With increasing age, the chick's requirement for potassium was observed to decrease.

NOTES

Letters to the Editor

Dear Sir:

I was very interested that a recent article in *Nutrition Reviews* emphasized the role of protein in the development and treatment of some of the anemias we have in Ghana (*Nutrition Reviews* **17**, *193* (*1959*)).

Dietary iron deficiency is not at all common in this area and it is felt that the terms hypochromic anemia and "iron deficiency anemia", which was rightly put into quotes, should not be used interchangeably. Clinicians and field workers here as elsewhere in the tropics recognize the fact that huge loads of hookworm may exist without any serious anemia and the clinical severity of such an infection depends on the state of nutrition of the individual. In Ghana this often means the extent of protein malnutrition present.

It is my experience that removal of the hookworms and iron therapy alone never achieve fully successful results. For outpatient therapy such patients always benefit from an addition of skimmed milk to the diet. This agrees with the findings of Stanier and Holmes (*Brit. J. Nutrition* **8**, *155* (*1954*)). The role of protein in the treatment of hookworm anemia has not been separately recognized in the past because reporters have ignored the drastic improvement in the diet of such a patient when he goes onto hospital food.

In this area, protein malnutrition and other nutritional deficiencies take a severe toll of infant and child life. In the first year of life, malaria is probably more important since the infants are often breast fed satisfactorily. Bruce Chewatt halved the infant mortality in Kilaro by controlling malaria.

Among the small children of one to four, however, the situation is different. This is the age during which some immunity to malaria has already been established. Malnutrition, which in Ghana means protein malnutrition, is the usual primary or secondary cause of death. From the regions where data exist, the ratio of deaths of children aged one to four to that of infants is between 0.6 and 0.8 (1957). The figure for Britain has dropped from 0.66 in 1870–72 to 0.155 in 1955. This ratio has been suggested by Wills and Watkins (1958), quoted by Platt as a good index of malnutrition for communities where vital statistics are not good. Post mortem findings in Accra certainly support the suggestion that malnutrition is one of the most important causes of death in young children.

The relationship of protein malnutrition to anemia in tropical regions has been excellently dealt with by Platt (*Proc. Nutr. Soc.* **15**, *103* (*1956*)).

F. T. Sai, M.R.C.P.E.
Medical Research Institute
Accra, Ghana

Dear Sir:

We were interested in the views recently expressed on the etiology and treatment of iron deficiency anemias in Ghana (*Nutrition Reviews* **17**, *193* (*1959*)).

Although we have no experience in Ghana, we should be surprised if the iron deficiency anemias there are in any way different from those described in nearly all other parts of the tropical world and which have been shown again and again to respond to oral iron administration and in most cases to be associated with considerable intestinal blood losses due to hookworm infection, which can reach 200 or more ml. per day with worm loads of up to 2500 adult worms, of which 40 to 70 per cent are *Ancylostoma duodenale*. Blood and iron losses have been estimated using radio-isotope techniques (Roche *et al.*, 1957; Foy and Kondi, 1958, in press 1959).

These blood losses stop with the removal of the hookworms. Treatment with oral iron results in a rise in the hemoglobin level to

normal in the majority of cases. Removal of the hookworms will not result in any improvement of the blood picture unless iron is also given. This is to be expected since removal of the worms is unlikely to replace exhausted or diminished iron stores. Unless, however, the worms are removed, anemia will sooner or later recur once iron therapy is stopped. The marrows in these iron deficiency anemias are erythronormoblastic, active and sometimes have giant stab-cells, the significance of the latter not being fully understood (Foy and Kondi, in press 1959). These anemias have been shown to be typically iron deficient, having low mean corpuscular hemoglobin concentrations, absence of marrow iron, high unsaturated iron binding capacities and low serum iron, and hemoglobin values in most cases between 2 and 8 g. per 100 ml.

These anemias are due to a specific deficiency of iron, and it is to be expected that they would respond to iron. In no instance have we found them to respond to protein alone. In rare cases there may be a slightly more rapid response to iron and protein combined; so far as we know there is no evidence that iron deficiency anemias respond to protein alone.

Regarding the mortality among children in Ghana, is not this to be anticipated in an area of high incidence of malaria and in an age group which has not yet acquired the "immunity" characteristic of the adult African who has been exposed to malaria?

The anemia associated with acute or chronic malaria is not iron deficient and responds in the great majority of cases to anti-malaria treatment unless associated with hookworms, in which case iron will also be necessary.

Other factors may also enter into the etiology of iron deficient anemias in the tropics such as deficiency of iron in the diet, poor absorption of iron on account of the bulk and high phytate and phosphate and low calcium content, which may adversely affect absorption. Excessive dermal losses associated with high perspiration and cell desquamation rates may also be a factor.

If it can be established that the Ghana iron deficiency anemias do indeed respond to dried skimmed milk, we should like to be assured that the milk really had no iron in it. Walker (1956) has shown that food and fermented liquors prepared in iron utensils have a very high iron content, and we should like to know whether the skimmed milk taken in Ghana was prepared in iron utensils and whether it had any iron in it.

We feel that it is imperative to emphasize that the iron deficiency anemias of the tropics are most unlikely to respond to the administration of dried skimmed milk which is devoid of iron, as suggested in your article.

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ATHENA KONDI, M.D.
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Recent Books

Human Nutrition and Dietetics. Sir Stanley Davidson, A. P. Meiklejohn and R. Passmore. Foreword by Lord Boyd Orr. E. & S. Livingstone Ltd., Teviot Place, Edinburgh. Pp. 856. Price 84s.

Food. The Yearbook of Agriculture. U.S. Department of Agriculture. For sale by the Superintendent of Documents, Washington 25, D.C. Pp. 736. Price $2.25.

History of the American Dietetic Association 1917–1959. Edited by Mary I. Barner. Published by J. B. Lippincott Co., Philadelphia, Pa., and Montreal, Canada. Distributed in Great Britain by Pitman Medical Publishing Co., Ltd., London. Pp. 312.

Erratum

In the August 1959 issue under Recent Books the number of pages given for *Proteins in Foods* by S. Kuppuswamy, M. Srinivasan and V. Subrahmanyan (Indian Council of Medical Research) should have been 290.

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